

Effect of Fermentation and Bacterial Inoculation on Lucerne Cell Walls

Beth A Jones,^a Ronald D Hatfield^{a,*} and Richard E Muck^b

^aDepartment of Agronomy, University of Wisconsin and ^bUS Dairy Forage Research Center, USDA, Agricultural Research Service, 1925 Linden Drive West, Madison, Wisconsin 53706, USA

(Received 14 November 1991; revised version received 21 May 1992; accepted 7 July 1992)

Abstract: Changes were found in the cell wall composition of lucerne after ensiling at three different dry matter (DM) contents. The amount of protein associated with cell walls was reduced during ensiling, regardless of inoculation level, by 46–68%, whereas protein associated with the lignin residue was reduced to a lesser extent (< 40%). The effect of ensiling on individual sugars of the cell wall varied. Uronic acids of the cell wall were decreased by 12% with wilted silages (290 g DM kg⁻¹) but were unchanged in a higher dry matter silage (401 g DM kg⁻¹) and silages with a limited pH change during ensilage. The arabinose and galactose contents, as a fraction of cell walls, decreased (15–24%), increasing glucose and xylose contents proportionally. Inoculation decreased arabinose and galactose contents early in the fermentation when the pH decline was enhanced but final values were not significantly different ($P > 0.32$). Removal of the sugars from the cell wall appears to be related to pH because silages with little pH change had no change in the cell wall sugars and inoculation reduced the cell wall sugars only after silage pH declined.

Key words: lactic acid bacteria, fiber, neutral detergent fiber, ensilage, *Medicago*.

INTRODUCTION

Ensiling of lucerne is a major conservation method for the production of ruminant feeds in the US. Changes in the cell walls of lucerne during ensiling may alter the feed value of the silage. Research on grasses has demonstrated hemicellulose to be degraded by both plant enzymes and acid hydrolysis (Dewar *et al* 1963; Morrison 1979). This degradation during ensiling has resulted in reduced neutral detergent residue of grass silage as compared to initial herbage (Chestnut *et al* 1988; Spoelstra 1990). A study evaluating the fermentation effect on the cell walls of wilted lucerne observed a different effect (Morrison 1989). Morrison (1989) reported a small reduction in the sugars comprising the hemicellulose fraction but observed a greater reduction in cellulose. The discrepancy of the ensiling effect on cell walls of grasses and lucerne was attributed to difference in the cell wall structure. However, studies utilizing lucerne hay and silages (30–35% DM) suggest that the hemicellulose fraction

(determined by neutral-detergent fiber (NDF) residue minus acid-detergent fiber (ADF) residue) is degraded during ensiling, but cellulose (ADF residue minus lignin residue) is not degraded (Nelson and Satter 1990). Further clarification of the fermentation effect on cell wall components of lucerne is required.

In a summary of studies where bacterial inoculants significantly affected the fermentation of grass and lucerne, DM digestibility was enhanced ($P < 0.10$) in 53% of the trials (Muck and Bolsen 1991). The arabinose residues are acid labile (Timell 1965; Morrison 1979) and reduced substitutions of arabinose on xylans have increased rumen degradation of xylans (Brice and Morrison 1975). This suggests that the effect of inoculation on digestibility or cell wall composition could be due to a lowered pH, enhancing acid hydrolysis of arabinose.

Results reported in this paper show the effect of ensiling and inoculation on cell walls of lucerne ensiled at different dry matter contents.

* To whom correspondence should be addressed.

EXPERIMENTAL

Ensiling of lucerne

Experiment 1

Lucerne (*Medicago sativa*) was grown in a greenhouse, harvested at bud stage and manually chopped to 2.5 cm. To ensure the presence of lactic acid bacteria, the chopped lucerne was inoculated with lactic acid bacteria and ensiled into laboratory silos (sterile 100 ml polypropylene tubes) according to the method of Muck (1987). The inoculum was a mixture of *Lactobacillus plantarum* and *Pediococcus cerevisiae* (Chris Hansen's Laboratory, Milwaukee, WI, USA). Silos were incubated in a 30°C water bath for 1, 2, 4, 8 and 56 days before replicate silos were removed, frozen and stored at -20°C.

Experiment 2

Field-grown lucerne was harvested at bud stage in October prior to frost damage and wilted in the field to 290 and 401 g DM kg⁻¹ herbage. Wilted lucerne was hand chopped, inoculated and ensiled as previously described. Inoculum was applied at two rates, a low rate (2×10^2 colony forming units (CFU) g⁻¹ lucerne) to insure a low level of lactic acid bacteria and a higher rate (6×10^6 CFU g⁻¹ lucerne). Silos were incubated at 30°C and replicate silos were pulled after 1, 2, 4, 8, 16, 32 and 64 days of ensiling.

Inocula and uninoculated lucerne extracts were plated on Rogosa agar and incubated anaerobically for enumeration of lactic acid bacteria (Muck 1989).

Analyses

A 25-g sample removed from each silo was diluted with distilled water (1 g:10 ml), macerated in a blender for 30 s, and filtered through two layers of cheesecloth. Analysis of the fermentation characteristics of the silage were completed on the water extract filtrate. A pH reading was recorded immediately while organic acids (Muck 1990), non-protein nitrogen (NPN), ammonia nitrogen (NH₃-N) and free amino acid nitrogen (FAA-N) (Muck 1987) were determined on the filtrate after freezing.

The residues were freeze-dried and ground through a 1 mm screen using a Udy mill. Cell wall material was suspended in potassium phosphate buffer (10 mM + 0.01 % NaN₃) and heated for 1 h in a boiling water bath. Samples were cooled to 60–65°C, 10 units of α -amylase (Sigma A3403) added, and placed in a 55°C waterbath for 2.5 h. Following α -amylase treatment, cell wall material was washed with ethanol followed by acetone (Hatfield 1991) and cell walls collected by filtration. Total nitrogen content of the cell wall was determined

using the method of Hach *et al* (1985) with the digestion procedure of Brotz and Schaeffer (1984). Cell wall material was hydrolyzed on ice in 72 % sulfuric acid for 30 min followed by a 2 h incubation at room temperature. Hydrolyzates were diluted to 1.6 M sulfuric acid, capped and heated in an oven at 100°C for 3 h. After dilution and prior to heating, an aliquot (200 μ l) was removed for determination of total uronics (Blumenkrantz and Asboe-Hansen 1973). After heating, internal standards (7.5 mg inositol, 5 mg 2-deoxyglucose) were added to the hydrolyzates which were then filtered through a glass filter (1.2 μ m) to collect the insoluble residue which gives an estimate of lignin. Nitrogen (using the above procedure) and ash (4 h at 500°C) contamination of lignin residues were determined. The filtrates (1.0 ml) were neutralized with BaCO₃, pelleted by centrifugation and the solution filtered through a glass filter (1.2 μ m).

In experiment 1 structural polysaccharides solubilized by heating during the amylase treatment were recovered after dialysis against distilled water for 24 h and analyzed for uronics and neutral sugars (Dubois *et al* 1956). The neutral sugars in the neutralized filtrate were estimated by gas chromatographic separation of their alditol acetates using inositol as the internal standard (Blakeney *et al* 1983). For experiment 2, polysaccharides solubilized by heating during the amylase treatment were precipitated in 90 % ethanol. The insoluble material was pelleted by centrifugation and solubilized in water before determination of total uronics and neutral sugars. Lignin residues were determined as indicated previously. Total uronics were estimated on the diluted hydrolyzate while neutral sugars were determined by high-performance liquid chromatography (HPLC; Dionex Carbohydrate System) with 2-deoxyglucose (5 mg per sample) as the internal standard. Ions (mainly Ba²⁺, CO₃²⁻, SO₄²⁻) were removed from the neutralized filtrates by passing through cation (Dowex 50W-X8 H⁺) and anion (Supelclean LC-SAX Supelco) exchange columns wetted with distilled water. All samples were adjusted to an acidic pH by the addition of 10 μ l of 1 M acetic acid prior to passing through the anion exchange columns. Samples were loaded onto a Dionex Carbohydrate column (HPIC ASG, 10 μ m, 5 mm \times 30 cm) at a solvent flow of 1 ml min⁻¹. Four minutes after injection the solvent (0.01 M NaOH) was changed to deionized water and elution continued for a further 27 min. Individual sugars were detected and quantified by a pulse amphoteric detector. Total recoveries of the neutral sugars, lignin, uronics and protein were 95–101 % of the starting material for both experiments.

The unensiled lucerne was ground (1 mm) and analyzed for buffering capacity (Muck 1990), total nitrogen and total water soluble carbohydrates (Dubois *et al* 1956).

TABLE 1
Characteristics of lucerne prior to ensiling

Dry matter (g kg ⁻¹ forage)	Crude protein (g kg ⁻¹ DM)	Soluble sugars (g kg ⁻¹ DM)	Buffering capacity (meq kg ⁻¹ DM)
195	252	43.5	488
290	207	54.8	457
401	217	61.2	447

Statistics

The effects of inoculation and ensiling were analyzed separately. Inoculation effect was evaluated at 1, 8 and 64 days of ensiling with the data from experiment 2. The effect was determined within each dry matter content and days ensiled using the model

$$X_{ij} = \mu + v_i + \varepsilon_{ij}$$

where X_{ij} is the observation of the i th treatment and j th silo, μ is the population mean, v_i is the effect of the i th treatment, and ε_{ij} is the error associated with the i th treatment and j th silo.

The effect of dry matter content and days ensiled were not determined due to their known effect on silage fermentations (Pitt *et al* 1985; Muck 1987, 1990).

Fermentation effects were determined by comparing the values to the unensiled lucerne with the end-point of the ensiling period. Data from experiments 1 and 2 were used. Data in experiment 2 were grouped across inoculation rate since treatment means for cell wall components were not significantly different at 64 days of ensiling ($P \geq 0.31$). Fermentation effect was determined within each DM content using the above model.

RESULTS AND DISCUSSION

Fermentation characteristics

Characteristics of the unensiled lucernes are shown in Table 1. The lucerne had typical values for soluble sugars and buffer capacity (Melvin 1965; Muck and Walgenbach 1985; Jones *et al* 1991) implying that the lucerne used in experiment 1 and the 290 g DM kg⁻¹ lucerne used in experiment 2 would be limited for fermentable substrates (Melvin 1965; Jones *et al* 1992). Epiphytic lactic acid bacteria on the lucerne were < 50 CFU g⁻¹ for all DM. Inoculation increased the number of lactic acid bacteria up to 2.8×10^4 CFU g⁻¹ herbage in experiment 1, and 676 and 5.50×10^6 CFU g⁻¹ herbage for the low inoculation (LI) and high inoculation (HI) treatments, respectively, in experiment 2. The numbers of bacteria present in LI are typical of the

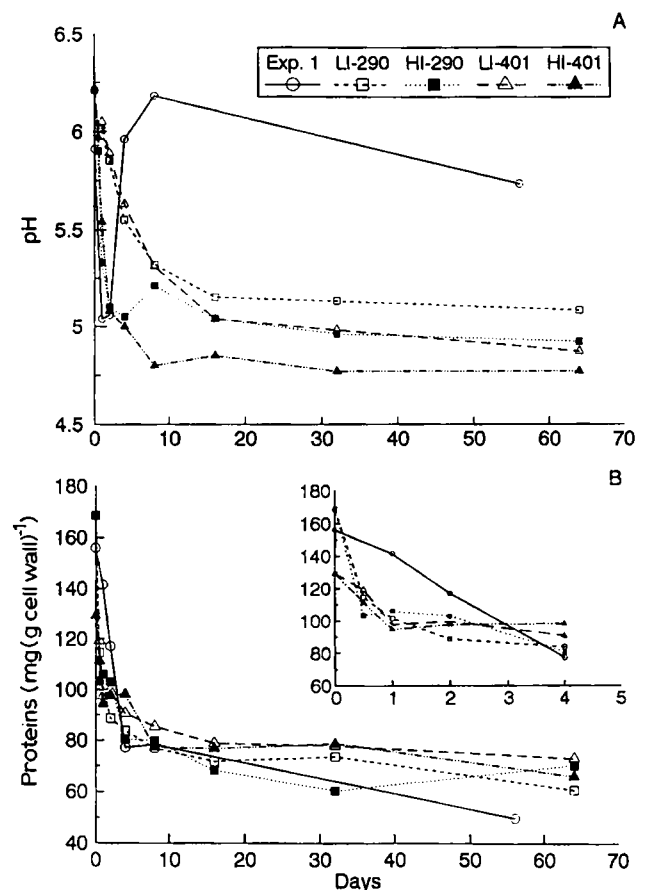


Fig. 1. Effect of ensiling on pH and protein associated with the cell wall fraction of lucerne at different DM values. (A) pH decline (B) Decline of the protein associated with the cell wall fraction of ensiled lucerne. Figure insert depicts decline of protein during the early stages of the fermentation. Legend: Exp 1, lucerne ensiled as fresh cut herbage (195 g DM kg⁻¹), LI-290, wilted lucerne (290 g DM kg⁻¹) inoculated at a low rate (200 CFU (g lucerne)⁻¹) and ensiled; HI-290, wilted lucerne (290 g DM kg⁻¹) inoculated with a high rate (6×10^6 CFU (g lucerne)⁻¹) and ensiled; LI-401, wilted lucerne (401 g DM kg⁻¹) inoculated at the low rate and ensiled; and HI-401, wilted lucerne inoculated at the high rate and ensiled.

numbers present on lucerne prior to ensiling (Muck 1989).

The pH decline in the silages is shown in Fig. 1A. Lucerne ensiled fresh had insufficient fermentable substrates to maintain a homolactic fermentation and low pH (see Table 4). As a result of the high pH, there

TABLE 2
Fermentation characteristics of lucerne silages

Dry matter (g kg ⁻¹)	Inoculation level ^a	Days ensiled	pH	Silage parameter ^b				
				NH ₃ -N (g kg ⁻¹ total N)	FAA-N (g kg ⁻¹ total N)	NPN (g kg ⁻¹ total N)	Lactate (g kg ⁻¹ DM)	Acetate (g kg ⁻¹ DM)
195	NA	2	5.06	57	305	622	44.8	11.9
		56	5.73	223	491	918	22.8	52.7
290	LI	2	5.85*	33	239	553	17.0*	13.5
	HI	2	5.10 (0.027) ^c	39 (2.9)	229 (5.9)	550 (16.0)	37.3 (1.27)	9.7 (2.30)
	LI	64	5.08	142	335*	713	50.1	44.7
	HI	64	4.92 (0.093)	130 (12.8)	402 (23.2)	788 (63.9)	56.2 (4.73)	31.0 (4.95)
401	LI	2	5.89	17*	213	498*	4.7*	5.9
	HI	2	5.08 (0.035)	20 (0.8)	210 (2.67)	415 (1.9)	29.9 (1.1)	4.5 (0.81)
	LI	64	4.87	107	334	599	53.9	25.2
	HI	64	4.77 (0.038)	95 (10.7)	340 (7.1)	553 (31.1)	51.0 (2.53)	23.0 (2.15)

^a Treatments were LI—low inoculation rate, HI—high inoculation rate, NA—not applicable.

^b Nitrogen parameters are NH₃-N—ammonia nitrogen, FAA-N—free amino acid nitrogen, NPN—non-protein nitrogen.

^c Square root of the standard error of mean (SEM). No statistics were completed on the 195 DM kg⁻¹ silages.

* $P < 0.05$.

TABLE 3
Influence of rate of inoculation on cell wall components of lucerne silages

DM	Inoculation rate	Days ensiled	Cell wall component (mg g ⁻¹ cell wall DM)				
			Lignin	Arabinose	Galactose	Glucose	Xylose
290	LI	1	175	39.6	25.1	357	107
	HI	1	174 (11.2) ^a	38.3 (0.62)	24.9 (0.30)	361 (15.1)	108 (4.0)
	LI	8	175	38.4*	25.2	362	111
	HI	8	179 (7.8)	34.4 (1.21)	23.0 (2.55)	370 (19.3)	112 (9.1)
	LI	64	171	30.3	21.1	368	113
	HI	64	177 (9.0)	31.5 (3.14)	22.4 (1.67)	363 (6.4)	110 (4.7)
401	LI	1	175	40.3*	24.7	376	111
	HI	1	185 (8.9)	37.8 (0.27)	24.1 (1.00)	377 (10.4)	116 (3.3)
	LI	8	175	37.3*	25.3*	375	107
	HI	8	173 (7.0)	33.9 (0.32)	23.1 (0.40)	379 (7.9)	115 (3.7)
	LI	64	179	30.8	22.8	376	107
	HI	64	176 (4.3)	32.5 (1.19)	22.1 (0.96)	371 (5.3)	113 (3.5)

^a Square root of the standard error of mean (SEM). Statistical comparisons were made between inoculation rates within days ensiled and DM.

* $P < 0.05$.

TABLE 4
Effect of fermentation on the cell wall composition of lucerne

DM	Days ensiled	Protein contamination (mg g ⁻¹)		Cell wall component ^a (mg g ⁻¹ cell wall)								
		Cell wall material	Lignin residue	Lignin	Uronics	Fucose	Rhamnose	Arabinose	Galactose	Glucose	Xylose	Mannose
195	0	157**	193**	166	120	4.9	19.0	44.6	25.8	364	83	19.3
	56	50	119	168	123	5.1	19.6	47.2	26.1	365	88	22.3
		(5.7) ^b	(5.5)	(8.2)	(2.7)	(0.46)	(5.41)	(12.76)	(0.73)	(8.1)	(9.8)	(4.27)
290	0	169**	145**	170	120*	2.9	13.6*	40.2**	27.5*	357	104	23.5
	64	65	94	168	114	2.6	11.3	30.5	21.7	365	112	22.5
		(3.1)	(3.1)	(7.6)	(2.0)	(0.18)	(0.94)	(2.47)	(1.26)	(13.6)	(3.6)	(0.89)
401	0	129**	147**	163	117	2.8	11.6	37.6*	26.0*	360	111	23.6
	64	70	134	167	119	2.6	11.9	31.6	22.4	374	111	22.1
		(5.9)	(3.0)	(4.2)	(1.9)	(0.15)	(1.28)	(1.23)	(1.19)	(11.6)	(5.5)	(0.91)

^a Cell wall weights were corrected for contaminating protein.

^b Square root of the standard error of mean (SEM).

** $P < 0.01$, * $P < 0.05$ between initial forage and final silage within each DM content.

was extensive proteolysis and deamination. Butyric acid concentration of the fresh silage was $< 5 \text{ mg kg}^{-1}$ DM ruling out a clostridial fermentation. Fermentation of the wilted lucernes, 290 and 401 g DM kg⁻¹, resulted in a typical pH decline (Fig 1A) with the HI resulting in a faster pH decline and higher lactic acid contents early in the fermentation ($P < 0.05$), but the final silage characteristics were not different ($P \geq 0.12$) (Table 2). The limited response of the higher rate of inoculation on final characteristics could be due to insufficient substrate (Jones *et al* 1991), but most likely caused by the dominance of the inoculant bacteria in both the LI and HI treatments.

Changes in cell wall components

The proteins associated with the cell wall material have not previously been monitored during ensiling. The actual amount of these proteins will be dependent on the fractionation scheme and declines during ensiling (Fig 1B). More than 50 mg crude protein per gram cell wall were lost from the silages during ensiling, with the 195 DM silages losing the most (107 mg g⁻¹ DM). Inoculation level had no effect on the extent of protein loss. Cell wall material, isolated from lucerne silages, must be corrected for the variation in protein content prior to comparisons on a weight basis.

The level of inoculation did alter the solubilization of arabinose and galactose from cell walls of lucerne silages but the effect was lost at the fermentation end-point (Table 3). The increased solubilization seen during the early stages in the fermentation corresponds with the effect of inoculation on pH decline suggesting that the solubilization is related to pH and not plant enzymes. Of the cell wall neutral sugars, arabinose is more sensitive to

acid hydrolysis (Timell 1965). Loss of galactose from the cell wall is due to its linkage with arabinose (Hatfield 1991). Thus, as arabinose is acid hydrolyzed, galactose is lost. Solubilization of arabinose may alter digestibility of the cell wall and consequently the silage (Brice and Morrison 1982). Xylans from maturing grass were more digestible when arabinose substitution was reduced (Brice and Morrison 1982), although this has not been demonstrated with lucerne. There was no effect ($P \geq 0.32$) of inoculation rate on the following cell wall components: lignin nitrogen, protein, hot-water-soluble polysaccharides, fucose, mannose, rhamnose, and total uronic content (data not shown).

Fermentation altered the cell wall components when comparing initial composition of the cell wall with the end-point of the fermentation (Table 4). Protein associated with the cell wall and lignin were decreased by fermentation. Proteolytic cleavage of these proteins after solubilization is unknown but is likely considering the amount of soluble non-protein nitrogen that was present in these silages (Table 2). Cell wall polysaccharides soluble in hot water were also reduced by fermentation (data not shown). Uronic concentration in these polysaccharides was reduced by 0.26–0.62 mg g⁻¹ silage DM, a 15–27% reduction; whereas, the concentrations of neutral sugars were reduced to a greater extent (38–59%) releasing 1–4 mg g⁻¹ silage DM.

Composition of the cell wall sugars released during acid hydrolysis changed during ensiling, with extent of change varying with silage dry matter and pH (Table 4). Lucerne ensiled at 195 DM resulted in no changes in the concentration of cell wall sugars. This is not surprising considering that the final pH of the silage was 5.7. Little acid hydrolysis of cell wall sugars could occur with only a decrease in pH of 0.3 units.

Cell walls from wilted lucerne responded differently to fermentation (Table 4). Arabinose and galactose decreased as a component of cell walls at both 290 and 401 g DM kg⁻¹, while rhamnose decreased only at 290 DM. Loss of these sugars along with a decrease in cell wall uronics (290 g DM kg⁻¹) suggests that pectin fractions of the cell wall are being affected (Hatfield 1990, 1991). Cell wall proportion of glucose and xylose at 290 DM and glucose at 401 DM increased as a result of other cell wall sugars decreasing. The hydrolysis of arabinose, galactose and rhamnose released 18 and 10 mg of sugar per gram cell wall of lucerne silage at 290 and 401 g silage DM, respectively. This converts to 8.3 and 4.5 mg non-cellulosic sugars released per gram silage DM (460 and 450 g cell wall kg⁻¹ DM for 290 and 401 DM silages, respectively). The selective hydrolysis of non-cellulosic sugars due to ensiling corresponds to work with grasses (Dewar *et al* 1963; Morrison 1979) however, previous work with lucerne is varied. Morrison (1989) measured a decrease in glucose of the cellulosic fraction and in xylose of hemicellulose during ensiling of lucerne that was not found in this study. Reasons for these discrepancies are not apparent but could be related to the low fiber recoveries obtained in the earlier study. Morrison (1989) did obtain the greatest reduction in arabinose of the hemicellulose and pectin fractions during ensiling which corresponds to the present study. The additional loss of galactose and uronics in this study suggests the sugars are being lost from the pectin fraction of lucerne (Darvill *et al* 1980; Hatfield 1990). Arabinose in pectin arabinans and arabinogalactans is predominately in the acid-labile furanose form and could be acid hydrolyzed during ensiling (Darvill *et al* 1980). The present results do concur with data that suggests that neutral detergent residues of ensiled lucerne were selectively hydrolyzed (Nelson and Satter 1990). However, since the greatest change in cell wall components during ensiling in this study occurred with protein associated with the cell wall fraction, changes in protein content of the detergent fiber residues must be checked.

CONCLUSIONS

Cell wall components of lucerne were altered during ensiling. Protein associated with cell wall carbohydrates and lignin was significantly reduced during ensiling. This reduction of protein must be accounted for when evaluating cell walls or fiber components between lucerne hays and silages or ensiling treatments. The cell wall sugars arabinose, galactose, rhamnose and uronics were decreased by 4–24% with the extent varying according to DM, sugar type and pH decline. The effect of increasing the level of inoculation on altering the cell walls of silages appears to be that of enhancing the pH decline which results in acid hydrolysis of the cell wall.

Additional research is required to validate these fermentation effects on lucerne cell wall material across lucerne varieties and growing conditions.

REFERENCES

- Blakeney A B, Harris P J, Henry R J, Stone B A 1983 A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr Res* **113** 291–299.
- Blumenkrantz N, Asboe-Hansen G 1973 New method for quantitative determination of uronic acids. *Anal Biochem* **54** 484–489.
- Brice R E, Morrison I M 1982 The degradation of isolated hemicelluloses and lignin–hemicellulose complexes by cell-free, rumen hemicellulases. *Carbohydr Res* **101** 93–100.
- Brotz P G, Schaeffer D M 1984 Nitrogen, calcium and phosphorus determinations from a single digestion of feed or feces. *J Anim Sci* (Suppl 1) **59** 408.
- Chestnut A B, Berger L L, Fahey G C Jr 1988 Effects of conservation methods and anhydrous ammonia or urea treatments on composition and digestion of tall fescue. *J Anim Sci* **66** 2044–2056.
- Darvill A, McNeil M, Albersheim P, Delmar D P 1980 The primary cell walls of flowering plants. In: *The Biochemistry of Plants*, Vol 1, ed Stumpf P K, Conn E E. Academic Press, New York, USA, pp 91–163.
- Dewar W A, McDonald P, Whittenbury R 1963 The hydrolysis of grass hemicelluloses during ensilage. *J Sci Food Agric* **14** 411–417.
- Dubois M, Gilles K A, Hamilton J K, Rebers P A, Smith F 1956 Colorimetric method for determination of sugars and related substances. *Anal Chem* **28** 350–356.
- Hach C C, Brayton S V, Kopelove A B 1985 A powerful Kjeldahl nitrogen using peroxydisulfuric acid. *J Agric Food Chem* **33** 1117–1123.
- Hatfield R D 1990 Carbohydrate composition of alfalfa cell walls isolated from stem sections differing in maturity. *J Agric Food Chem* **40** 424–430.
- Hatfield R D 1991 Alfalfa-stem pectins: enzymatic degradation and structural characterization of a buffer-soluble fraction. *Carbohydr Res* **212** 177–186.
- Jones B A, Satter L D, Muck R E 1992 Influence of bacterial inoculant and substrate addition to alfalfa ensiled at different dry matter contents. *Grass Forage Sci* **47** 19–27.
- Melvin J F 1965 Variations in the carbohydrate content of lucerne and the effect on ensilage. *Aus J Agric Res* **16** 951–959.
- Morrison I M 1979 Changes in the cell wall of laboratory silages and the effect of various additives on these changes. *J Agric Sci (Camb)* **93** 581–586.
- Morrison I M 1989 Influence of some chemical and biological additives on the fibre fraction of lucerne on ensilage in laboratory silos. *J Agric Sci (Camb)* **111** 35–39.
- Muck R E 1987 Dry matter level effects on alfalfa silage quality I. Nitrogen transformation. *Trans ASAE* **30** 7–14.
- Muck R E 1989 Initial bacterial numbers on lucerne prior to ensiling. *Grass Forage Sci* **44** 19–25.
- Muck R E 1990 Dry matter level effects on alfalfa silage quality II. Fermentation products and starch hydrolysis. *Trans ASAE* **33** 373–381.
- Muck R E, Bolsen K K 1991 Silage preservation and silage additive products. In: *Field Guide for Hay and Silage Management in North America*, ed Bolsen K K. National Feed Ingredient Association, Des Moines, IA, USA, pp 105–126.
- Muck R E, Walgenbach R P 1985 Variations in alfalfa

- buffering capacity. ASAE paper no. 85-1535, ASAE, St Joseph, MI, USA.
- Nelson W F, Satter L D 1990 Effect of stage of maturity and method of preservation of alfalfa on production by lactating dairy cows. *J Dairy Sci* **73** 1800–1811.
- Pitt R E, Muck R E, Leibensperger R Y 1985 A quantitative model of the ensilage process in lactate silages. *Grass Forage Sci* **40** 279–303.
- Spoelstra S F 1990 Effects of cell wall degrading enzymes on silage composition at different ensiling conditions. In: *Proc Ninth Silage Conference*, Faculty of Agriculture, University of Newcastle upon Tyne, pp 27–28.
- Timell T E 1965 Wood hemicelluloses II. *Adv Carbohydr Chem* **20** 409–483.